

AN IMPROVED ASSAY OF CERULOPLASMIN ACTIVITY IN DOMESTIC SPECIES

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Ceruloplasmin is the major copper containing protein present in the plasma of animals. Activity of this enzyme is dependent on dietary copper, and provides an index of copper deficiency in domestic animals (Todd 1970; Bingley and Anderson 1972). Although p-phenylenediamine (PPD) is commonly used for the assay of ceruloplasmin activity (Ravin 1961; Bingley and Dick 1969), this substrate has several limitations. Schosinsky *et al.* (1974) described a method for measuring ceruloplasmin in human plasma, using o-dianisidine. This substrate appears to overcome many of the disadvantages associated with PPD. In the present study, the use of o-dianisidine for the assay of ceruloplasmin in a number of domestic species was investigated, and optimal assay conditions established for these species.

The standard assay consisted of adding plasma (0.05ml) to Na acetate buffer (0.75ml of 100mM, at the pH shown in table 1). After 5 min at 30°C the reaction was initiated by addition of o-dianisidine (0.20ml of 10mM o-dianisidine dihydrochloride in water). Duplicate incubations were performed: one was terminated 5 min after initiation and the second at the time shown in table 1, by the addition of sulphuric acid (2.0ml of 9M H₂SO₄). Activity was calculated from the difference in absorbances at 540nm (Schosinsky *et al.* 1974).

TABLE 1. Species ceruloplasmin properties and assay requirements.

| Species | Optimal buffer pH | o-dianisidine Km (mM) | Q ₁₀ (25/35) | Incubation Time (min) | Activity range (mU/ml) | n |
|---------|-------------------|-----------------------|-------------------------|-----------------------|------------------------|----|
| Ovine | 5.6 | 0.67 | 2.1 | 45 | 32-223 | 25 |
| Bovine | 5.7 | 1.20 | 2.2 | 45 | 9-151 | 26 |
| Caprine | 5.6 | 0.61 | 2.2 | 45 | 45- 82 | 6 |
| Equine | 5.6 | 0.55 | 2.3 | 45 | 38- 78 | 13 |
| Porcine | 5.3 | 1.30 | 2.0 | 15 | 196-409 | 16 |
| Servine | 5.6 | 0.49 | 2.3 | 45 | 58(pooled) | 5 |

For each species, optimal activity was observed when acetate buffer at the pH shown in table 1 was used. Higher concentrations of acetate (up to 250mM) had little effect on activity. Phosphate buffer (50mM) gave similar pH optima, but reduced activities by 4 to 15%. Maximal activities occurred with o-dianisidine concentrations in the assay of 2.0mM. Higher concentrations were inhibitory. At 2.0mM o-dianisidine, reaction rates were linear up to absorbances of at least 1.2. However non-linear enzyme dilution curves, attributed to inhibition of activity by chloride present in plasma, were observed for all species. Initial reaction lag periods of approximately 2 min were also observed.

The use of o-dianisidine offers a convenient means for measurement of ceruloplasmin activity in the species investigated. Its stability in aqueous solution (several months at 4°C) and stability of reaction products formed (>24 hrs at 25°C) are major advantages compared with PPD.

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